Using CO_2 for Biomanufacturing of Fuels and Chemicals

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Microbial cell factories offer an eco-friendly alternative for transforming raw materials into commercially valuable products because of their reduced carbon impact compared to conventional industrial procedures. These systems often depend on lignocellulosic feedstocks, mainly pentose and hexose sugars. One major hurdle when utilizing these sugars, especially glucose, is balancing carbon allocation to satisfy energy, cofactor, and other essential component needs for cellular proliferation while maintaining a robust yield. Nearly half or more of this carbon is inevitably lost as CO_2 during the biosynthesis of regular metabolic necessities. This loss lowers the production yield and compromises the benefit of reducing greenhouse gas emissions—a fundamental advantage of biomanufacturing.

Keywords: metabolic engineering ; CO2 fixation ; feedstock ; biomanufacturing ; electrochemical catalysis ; microbial electrosynthesis

1. Introduction

Carbon emission to our ecosystem and its accumulation in its highly oxidized state, carbon dioxide (CO_2), are the primary contributing factors to global climate change [1]. Since the 1960s, the total CO_2 emissions have rapidly increased, with a net annual escalation rate of 2.11% in recent years [2]. The push for carbon neutrality necessitates reimagining our feedstock sources. Over 90% of our chemicals and fuels are manufactured from fossil feedstocks, driving the need to transition toward a more circular industry model. G20 economies have implemented carbon emission taxes ranging from $3 to $60 per ton to incentivize CO_2 capture from industrial processes [3]. The cost of carbon capture varies based on the CO_2 source [4]. This suggests that, in some countries, obtaining CO_2 at zero cost may be possible. Therefore, exploring the potential of capturing and utilizing CO_2 is essential to mitigate the global warming challenge.

Photosynthesis is the natural way to capture CO_2 from the atmosphere and fix it into sugars or carbohydrates, which can then be used as the feedstocks for microbial cells to produce fuels and chemicals by green plants and algae. Therefore, biomanufacturing is considered more sustainable than chemical manufacturing with petroleum-based feedstocks. However, the production of biomass through the photosynthesis process still suffers the challenge of high-cost processing and low-energy efficiency [5]. While photosynthesis is a marvel of nature, its energy efficiency seldom surpasses 3%, constraining its industrial applicability. Moreover, using agricultural crops to provide feedstocks for biomanufacturing poses a sustainability challenge as it hinders food production and threatens biodiversity when natural areas are used for agricultural purposes.

Sugars such as glucose are the most widely used substrate for biomanufacturing in laboratory and industrial settings for historical and practical reasons. However, employing glucose may repress gene expression and specific biosynthetic pathways for certain biomanufacturing products. In most cases, glucose may also cause several limitations in cell metabolism, resulting in carbon loss as CO_2 [6]. This is particularly noticeable when the product of interest requires long synthetic routes from the starting carbon source when it has chemical properties distinct from the substrate or when unfavorable substrates are used, ultimately leading to low product yield [7].

Despite the predominant dependence of current industrial biomanufacturing processes on carbon-intensive carbohydrate substrates, including the C_5/C_6 sugars such as xylose and glucose derived from cellulosic biomass, it is worth acknowledging that the feedstock and raw materials significantly contribute to the overall cost of biomanufacturing [8]. Reducing the cost can be achieved by using more economical raw materials and designing new microbial cell factories that can efficiently utilize alternative feedstocks. Some microorganisms exhibit the inherent capability or possess the potential to metabolize C_1 and C_2 substrates [9]. These C_1 substrates, comprising CO_2, carbon monoxide (CO), methane (CH_4), methanol (CH_3OH), and formate ($CHOO^-$) [10], and C_2 substrates, comprising mainly ethanol and acetate [11], hold the gains of being inexpensive, naturally abundant, and straightforward manufacturing along with their abundant availability as by-products and industrial wastes [9]. Owing to the worldwide attention to continuous conversion of greenhouse gases, specifically CO_2 [12] to recover its diminished economic worth, scientists have a special interest in designing innovative CO_2 fixation methods with microbial entities, thereby assisting them in the synthesis of crucial substrate precursors (C_1 and C_2 chemicals) having the inherent capability to serve as biomanufacturing substrate in numerous processes [13][14].

However, the utilization of CO_2-derived C_1/C_2 chemicals for biomanufacturing is challenged by the inefficiency of conversion into desired bioproducts by native microorganisms, resulting in relatively lower productivity, limited energy availability, and deprived carbon yield, as compared with the utilization of C_5/C_6 sugars [14]. To address the associated challenges, major efforts have been made in the field of synthetic biology and metabolic engineering to evolve both natural microbes [15] and/or heterologous microorganisms by engineering the pathways or enzymes to improve their C_1 and C_2 substrate-utilizing capabilities [14][16][17][18][19]. Such interventions may range from enhancing native pathways to integrating entirely novel ones crafted from a deep understanding of metabolic networks and enzymology to improve carbon-fixation efficiency [19].

One of the pivotal concerns is the significant carbon loss, especially in the format of CO_2 during microbial fermentation [20][21], which comprises the advantageous of using biomanufacturing as one of the major efforts in reducing greenhouse gas emission [22]. Therefore, recycling the exhausted CO_2 back to the microbial fermentation process is also critical to the success of biomanufacturing.

2. Current Technologies Using CO_2 as a Feedstock for Biomanufacturing of Fuels and Chemicals

The conversion of CO_2 into value-added chemicals using microbes as biocatalysts is an exciting field of research with the potential to revolutionize biomanufacturing processes [23]. For using CO_2 as the feedstock for biomanufacturing, both one-step and two-step strategies can be applied. **Table 1** summarizes the general strategies for fixation of CO_2 for biomanufacturing. The one-step strategy uses the native or engineered pathways to directly fix CO_2 and convert it into desired fermentation products, typically with multiple carbons. Since CO_2 has the lowest energy format, producing high-value chemicals with a higher energy format requires extra energy; this can be achieved by either plants, algae, or cyanobacteria via a photosynthesis process that uses light as the energy source or other microorganisms with cofeeding higher energy-intensive chemicals such as hydrogen gas. The two-step strategy uses a hybrid electrochemical and biochemical conversion approach to fix CO_2 and convert it to the desired fermentation products at higher yield and efficiency, where the first step uses an electrochemical catalysis process to convert CO_2 into C_1/C_2 chemicals, followed by a second fermentation step to further convert C_1/C_2 chemicals into desired products by native or engineered microorganisms.

Table 1. General strategies for biotechnological fixation of CO_2.

Methods	Major Steps and Overall Reaction of CO_2 Fixation
One-step/Direct CO_2 fixation and conversion	• Calvin–Benson–Bassham (CBB) Cycle: $3CO_2$ + 12 ATP → GAP (→ ½ Glucose) • Wood–Ljungdahl Pathway (WLP): $2CO_2$ + CoA + $4H^+$ + $4e^-$ → Acetyl-CoA + $2H_2O$ • Reductive Glycine Pathway (rGlyP): $3CO_2$ + $3H_2$ → Pyruvate • Reductive Tricarboxylic Acid Cycle (rTCA): $2CO_2$ + CoA + 2ATP → Acetyl-CoA • 3-Hydroxypropionate (3HP) Bicycle: $2CO_2$ + 2ATP → Glyoxylate; CO_2 + Glyoxylate + ATP → Pyruvate • 3-Hydroxypropionate/4-Hydroxybutyrate (HP/HB) Cycle: $2CO_2$ (HCO_3^-) + CoA + 4ATP → Acetyl-CoA • Dicarboxylate/4-Hydroxybutyrate (DC/HB) Cycle: $2CO_2$ (HCO_3^-) + CoA + 3ATP → Acetyl-CoA
Two-step CO_2 fixation and conversion	Step 1 (electrochemical catalysis): CO_2 + H_2O + electricity → C_1/C_2 chemicals • CO_2 + $2H_2O$ + electricity → CH_3OH + $1.5O_2$ • CO_2 + H_2O + electricity → HCOOH + $0.5O_2$ • $2CO_2$ + $3H_2O$ + electricity → C_2H_5OH + $3O_2$ • $2CO_2$ + $2H_2O$ + electricity → CH_3COOH + $2O_2$ • CO_2 + electricity → CO + $0.5O_2$ • CO_2 + $2H_2O$ + electricity → CH_4 +$2O_2$ Step 2 (biomanufacturing): C_1/C_2 → biofuels and chemicals • Direct use of C_1/C_2: C_1/C_2 → fuels/chemicals + biomass • Cofeeding C_1/C_2 and C_5/C_6 sugars: C_1/C_2 + C_5/C_6 sugars → fuels/chemicals + biomass

2.1. One-Step Strategy—Direct Conversion

Internal carbon sequestration has taken many different forms throughout history. Even before the evolution of eukaryotic plants utilizing photosynthesis and light to convert CO_2 and energy from light to compose simple sugars, single-celled organisms had already developed mechanisms to capture atmospheric CO_2 and transform it into essential compounds for the cell's development. These primitive mechanisms, especially those in microorganisms like *Acetogens* and *Methanogens*, have been shown to be highly efficient in, utilizing unique proteins and metabolic pathways for carbon sequestration [1]. Furthermore, microorganisms, especially microalgae and cyanobacteria, exhibit significant advantages over higher plants in their capacity for CO_2 fixation as they can yield higher solar energy retention and the potential for year-round growth compared to their more complex plant counterparts [24]. While microalgae are well-recognized for their

CO_2 fixation capabilities, bacteria present advantages that cannot be overlooked [25]. Microalgae cultivation can be subject to biocontamination over prolonged use from fungal and bacterial species and often run into issues pertaining to even distribution of sun exposure over larger microalgae ponds due to their preferred growth environments, vastly limiting their ability to be utilized on an industrial scale without major alternations to the water infrastructure the microalgae is grown on. Bacteria and some yeasts, on the other hand, have been widely used in biotechnology industry due to their inherent compatibility to produce chemicals and their rapid growth rates and life cycles. Further, they are more inclined to accept DNA during genetic modification in the form of plasmids and genomic alternations. This ability allows bacteria and yeast to have DNA introduced into their cells of enzymes to complete metabolic pathways previously incompletely represented in the cells and allow production of specialized products, including bioalcohols and essential fatty acids. Through this biotechnological approach, CO_2 can be directly converted into value-added products, offering an advantage over traditional methods like catalytic conversion, which demand energy-intensive conditions [23].

2.2. Two-Step Strategy—Fixing CO_2 into C_1/C_2 Chemicals via Electrochemical Catalysis and Converting C_1/C_2 Chemicals into Bioproducts via Biomanufacturing

The two-step/indirect CO_2 fixation and conversion strategy takes the advantages of the current advances from both electrochemical CO_2 fixation into C_1/C_2 chemicals and the synthetic biology to further convert the derived C_1/C_2 chemicals into the fuels, chemicals, and pharmaceuticals via biomanufacturing process. A primary advantage of these substrates is their non-competitive nature with alimentary resources, which contributes to an economically sustainable framework while diminishing carbon efflux into the biosphere [26]. Nevertheless, it has been widely studied that the C_1/C_2 substrates can be produced from CO_2 via an electrochemical catalysis process [27], which uses renewable electricity from solar, wind, or hydraulic power to capture and fix CO_2 into specific C_1/C_2 products at high yield and selectivity. This two-step CO_2 fixation and conversion approach can potentially reduce the dependence on fossil oil-based fuels and chemicals and mitigate the impact of greenhouse gas emissions on the environment [28].

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